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Perspective of apple processing wastes as low-cost substrates for bioproduction of high value products: A review

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ABSTRACT

The fruit processing industries are experiencing surge due to the increasing demand of food products as a result of burgeoning human population. Apple and apple products are one of the major fruit and fruit products consumed all over the world. Apple processing industries generate huge quantities of solid and liquid sludge wastes. The solid residues consist of a mixture of skin, pulp and seeds derived from the production of concentrated apple juice, jam, and sweets and are collectively known as 'apple pomace'. Being highly biodegradable, the disposal of these wastes represents a serious environmental problem and presents many challenges. Often only 20% is retrieved as animal feed and the rest 80% goes to landfill, is incinerated or is sent to composting sites which results in release of greenhouse gases. However, advancement in technology has led to the alternative options of utilization of apple pomace. It can be used as a promising raw material for direct extraction of bioactive compounds and bioproduction of high value-added products, such as enzymes, organic acids, biofuels, among other products. This article reviews the work done for value-addition of this precious biomass which can help in setting up integrated process in the existing apple industries itself or separate small scale industries.

Contents

Abbreviations: AP, apple pomace; APS, apple pomace ultrafiltration sludge; BGL, β-glucosidase; CA, citric acid; CM, codling moth larvae; CTS, chitosan; GHGs, greenhouse gases; GRAS, generally recognized as safe; FDA, Foods and Drug administration; LA, lactic acid; TCA, tricarboxylic acid cycle; SmF, submerged fermentation; SSF, solid-state fermentation; SCP, single cell protein; WHO, World Health Organization; VAPs, value added products

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1. Introduction

Agro-based industries, especially apple processing industries, are experiencing a surge in their growth around the globe. This enormous increase in fruit processing has been generating million of tons of agro-industrial wastes worldwide. Every year, thousands of tons of apple pomace (AP) and apple pomace sludge (APS) are generated by apple processing industries in Canada. In 2008–2009, worldwide apple production exceeded 69,603,640 tons [Food and agriculture organization (FAO) of the United Nations, http://faostat.fao.org] out of which Canada contributed 455,361 tons (Table 1). This growth in turn has generated several million tons of wastes [25–30% solid pomace waste and 5–10% liquid sludge] during the processing of apple products, such as apple juice, jelly and cider, among others (Fig. 1). The direct disposal of agro-industrial by-products as a waste in the environment represents a major cause for environmental pollution and also an important loss of biomass which could be used for the production of different high value products. Nowadays, there is an increasing global trend towards the efficient utilization of natural resources. Sustainable food production and value-addition of wastes is the most important issue in the agro and food processing industries.

AP and APS being rich in carbohydrates and other vital nutrients and having high moisture content (70–75%, pomace), biodegradable organic load [high biological oxidation demand (BOD) and chemical oxidation demand (COD) values] are highly susceptible to microbial attack. For instance, the BOD of APS is 72,000 mg/l and the BOD to COD ratio is high (0.6), and AP has high COD value of 250–300 g/kg which indicates their high biodegradable nature [151,44]. It starts fermenting directly on the filter press during juice extraction. The wastes generated from the fruit processing industry cannot be directly dumped into the environment. These by-products must be managed properly in order to avoid their noxious effects. AP represents significant waste sources in many countries, as globally, several million tons of AP are generated annually. The management of agro-industrial wastes is a serious problem in the world [37,38].

Currently, these by-products are treated in traditional ways, such as landfilling, incineration, composting, low quality animal feed and land spreading. The dumping of these by-products can have several adverse impacts: (1) produce greenhouse gases (GHGs); (2) source of secondary pollution, such as emit foul smell due to microbial attack, and land spreading results in contaminated underground water table due to run off in rainy seasons; (3) negative effects on human health as the landfills and land spreading create breeding grounds for many human disease vectors which can cause epidemics; and (4) the industries incur losses due to treatment of waste and transportation costs for dumping into landfills, which is not cost-effective. Currently, only a small proportion of AP is utilized as a feed for the ruminants, added to soil as a fertilizer, and the large proportion of this inexpensive biomass goes to the composting sites and landfills, resulting in the release of GHGs and causing environmental nuisance and jeopardizing the health of people.

Nowadays, environmental biotechnology is emerging as a good option to tackle the adverse impacts of agro-industrial wastes. The use of agro-industrial wastes for bioproduction of valuable bio-products through microbial fermentation is economically important and can minimize various environmental hazards. AP and APS can be used as substrates for the microbial production of carboxylic acids, enzymes, biofuels, biopolymers and for the direct extraction of bioactive compounds, such as antioxidants.

Solid-state fermentation (SSF) also known as koji fermentation is gaining wide interest these days for the production of organic acids, enzymes and other biotechnological products [20,6,38–42,47,48]. Agro-industrial residues are generally considered the best substrates for koji fermentation processes, especially for

Table 1
Apple production and estimated waste generation in Canada and worldwide.

Producer	Apple production (tons)	Production (year)	Estimated production of waste (tons) (25–30% AP and 5–10 % APS)
Canada	455,361	2009	113,84–136,61 (AP) 22,768–45,536 (APS)
(Québec)	(116,088)		(29,022–34,826) (AP) 5804–11,609 (APS)
World	69,603,640	2008	17,400,910–20,881,092 (AP) 3,480,182–6,960,364 (APS)

Source: [15,39,172].

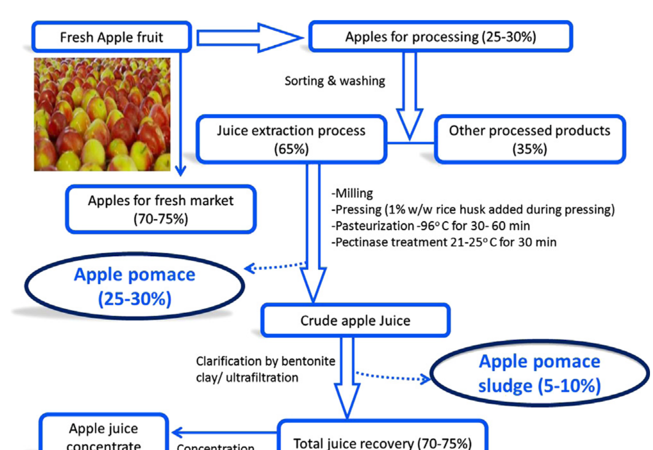


Fig. 1. Flowchart showing the production of AP and APS during processing of apples in the juice industry.

enzyme production [32,20,41,191]. The presence of lignin and cellulose/hemicellulose acts as natural inducers, and most of these residues are rich in sugar, promoting better fungal growth and thus making the process more economical especially for the cellulo- and ligninolytic enzymes [33,132,38].

AP has been used for the production of organic acids [153,39,40,48], aroma compounds [18,168,120], bioethanol [128,129,135], enzymes [63,41,42,43,47,103,175], edible mushrooms [182,54,187–187], edible fibers [72,114,135], pectin recovery [149], natural antioxidants [65,6], protein enriched animal feed [13,36,7,150,171], and insect diets [71] among others. AP has also been considered for environmental applications, such as textile dye removal [142] and as heavy metal absorbent [126]. APS has not been explored much except for the bioproduction of CA and insect diet [39,44,49,71].

In this context, this review article discusses the potential of apple industry wastes for the bioproduction of VAPs. The current biotechnological applications of AP and APS are discussed in detail. This article also discusses the direct extraction of bioactive compounds from apple industry wastes.

2. Proximate composition of apple processing wastes

Proximate composition of AP and APS is given in Table 2. Both AP and APS are rich sources of carbohydrates, minerals, vitamins and dietary fibers. These can be exploited for the bioproduction of organic acids, bioethanol, enzymes, pectin recovery, biocolor product and nutritional enriched animal feed and insect diet. Besides this AP also contains pectin making it a suitable substrate for the production of pectin esterase enzyme. APS can be used for the production of VAPs through submerged fermentation (SmF) which is an established method.

Recently, koji fermentation is also gaining global attention for the production of various biotechnological products [37]. Besides the efficient utilization of abundant lignocellulosic biomass, koji fermentation offers other marked advantages over SmF in terms of productivity, concentration of the products, lower effluent generation, low capital and recurring expenditures, reduced energy requirements, absence of foam formation, no sophisticated instrumentation requirements and high reproducibility. Among various microorganisms, filamentous fungi are widely employed in SSF due to their ability to grow efficiently on complex solid substrates and produce a wide range of extracellular products.

3. Biotechnological products derived from apple processing wastes

Various biotechnological commodities produced using AP and APS are given in Fig. 2. Both AP and APS show advantages as a raw material for biotechnological products, including (1) high content of polysaccharides (mainly cellulose, starch and hemicelluloses), (2) presence of mono-, di- and oligosaccharides, citric acid and malic acid, which can be metabolized by microorganisms and (3) richness in vitamins and other mineral ions which could limit the cost of nutrient supplementation for fermentation media.

3.1. Organic acids

3.1.1. Citric acid

The utilization of AP and APS for the bioproduction of organic acids, such as citric acid (CA) and lactic acid (LA), by various

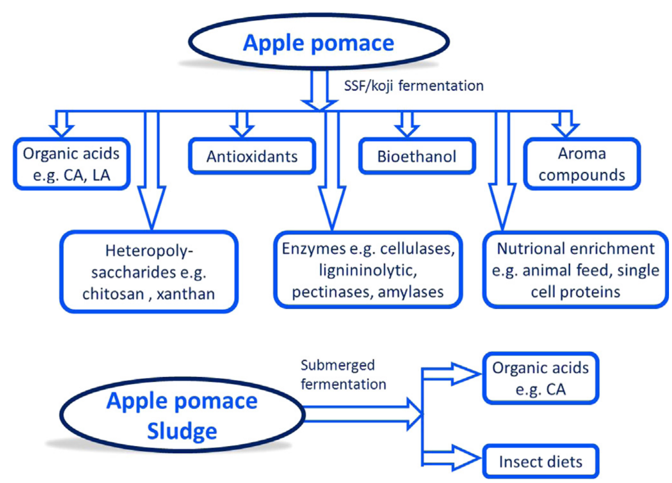


Fig. 2. Flowchart showing bioprocessing of AP and APS for various value added products.

Table 2
Proximate composition of AP and APS.

Biomass components	Composition (dry weight basis)		Micronutrients	Composition (dry weight basis) mg/kg otherwise mentioned	
	AP	APS		AP	APS
Initial pH	3.5 ± 0.1	3.3 ± 0.1	Al	–	259–304.6 ± 17
Total solids (g/l)	–	115–135 ± 5.0	Ca	0.06–0.1 **(%)**	912–1070 ± 58
Total nitrogen	6.8 (g/kg)	2.2–2.9 (g/l)	Cd	–	0.02–0.023 ± 0
Total carbon	127.9 g/kg	44.3–51.9 (g/l)	Cr	–	0.57–0.67 ± 0.03
Cellulose	7.2–43.6 (% w/w)	–	Cu	1.1	13.4–15.73 ± 1.3
Hemicellulose	4.26–24.40 (% w/w)	–	Fe	31.8–38.3	333–391 ± 56
Lignin	15.3–23.5 (% w/w)	–	K	0.4–1.0 **(%)**	6825–8012 ± 296
Pectin	3.5–14.32 (% w/w)	–	Mg	0.02–0.36 **(%)**	–
Total carbohydrates	48.0–83.8 (% w/w)	56.2–66 ± 1.7 (g/l)	Mn	3.96–9.0	–
Fiber	4.7–51.10 (% w/w)	–	Na	0.2 **(%)**	405–417 ± 45
Protein	2.9–5.7 (% w/w)	28.8–33.8 ± 2.0 (g/l)	Ni	–	–
Lipids (ether extract)	1.20–3.9 (% w/w)	5.1–5.9 (g/l)	P	0.07–0.076 **(%)**	–
Reducing sugars	10.8–15.0 (% w/w)	–	Pb	–	0.3–0.35 ± 0.03
Glucose	22.7	–	S	–	2200–2585 ± 157
Fructose	23.6	–	Zn	15.0	18.1–21.25 ± 2.5
Sucrose	1.8	–			
Arabinose	14–23	–			
Galactose	6–15	–			
Xylose	1.1	–			

microorganisms is given in Table 3. AP and APS have been utilized as an ideal substrate to cultivate different microorganisms for the production of CA [153,39,40,42,45]. Moreover, the pH of AP and APS is nearly 3–3.5 which is suitable for the bioproduction of CA by *A. niger* strains known to secrete high quantity of CA under acidic conditions [37,39,44]. Studies established that AP can be utilized as a substrate for CA production without any pretreatment and amendment with other costly synthetic nutrients [40,49,53]. CA concentrations of 342–365 g/kg dry AP were achieved through koji fermentation using AP with *A. niger* strains [40] as compared to the CA production achieved by other researchers using other agro-industrial wastes. Kumar et al. [107] carried out the CA production using AP with *A. niger* van. Tieghem MTCC 281 and achieved CA production of 46 g/kg dry AP with 4% (v/W) methanol at 30 °C after 5 days of incubation time.

Similarly, APS has been utilized first time for the bioproduction of CA [39,44]. Higher bioproduction of CA with 40.3 ± 2 g/l APS was achieved with optimum conditions of 25 g/l total solids (TS), 3% methanol as an inducer after 132 h fermentation time in a stirred fermenter [49].

CA is a multifarious carboxylic acid with high potential in the food, pharmaceuticals, cosmetics, detergents and agricultural sectors. CA is a natural metabolic intermediate, non-toxic, biodegradable, biocompatible and it is accepted worldwide as a GRAS (generally recognized as safe), as approved by the Joint FAO/WHO Expert Committee on Food Additives. Recently, CA has been used for various advanced applications, such as in biomedicine industry for synthesis of biopolymers for drug delivery, and culturing of wide variety of human cell lines; and nanotechnology, for bioremediation of heavy metals from soil and metal ore mines and for making water-based wood preservatives [37,46]. CA is considered as one of the important platform chemicals with numerous applications. However, reduction in prices, high energy and raw materials costs has turned once lucrative CA market into an unprofitable one. The search for inexpensive raw materials is vital to reduce the production cost of CA.

In this context, AP and APS represent ideal low cost substrates for the feasible and sustainable bioproduction of CA which can help to improve the overall cost economics and will stabilize the CA market. The simultaneous process through which waste fungal mycelium can be used for extraction of chitosan (CTS) as a co-product is an attractive alternative for stabilization of primary product i.e. CA prices [52]. Development of processes for extraction of CTS will lead to additional economic advantages to the CA industries.

3.1.2. Lactic acid

LA is a multifunctional and versatile organic acid having wide range of applications in the food, pharmaceutical, leather and textile industries and as a chemical feedstock. The global demand for LA is expected to be 2×10^5 tons/year by 2011 (Ramesh 2001) [140]. Industrial production of LA by chemical synthesis yields a racemic mixture of isomers not suitable for poly-LA synthesis. Alternative microbiological synthesis route exists that can yield pure isomers under suitable conditions. *Lactobacillus rhamnosus* can be cultured to produce L(+)−lactic acid of suitable chiral purity. Generally, glucose and sucrose are preferred carbon sources for LA production as compared to raw starchy substrates, such as barley, corn and wheat. However, on an industrial scale, the media needs to be supplemented with expensive nitrogen sources, such as yeast extract, peptone and vitamin solution, to sustain bacterial growth. Due to the high cost of raw material and nutrients and considering the market price of LA, the search for alternative, low-cost substrates and nutrient sources encompasses economic interest.

AP shows several attributes as a raw material for LA production, such as (1) high content of free sugars, comprising glucose and fructose, which are excellent carbon sources for LA production [83]; (2) high content of polysaccharides (cellulose, and hemicelluloses) which can be enzymatically hydrolyzed to give fermentable monosaccharides; (3) presence of other compounds (e.g. monosaccharides other than glucose and fructose, di- and oligosaccharides, citric acid, and malic acid) which can be metabolized by lactic bacteria [23]; (4) presence of metal ions (Mg, Mn, Fe, etc.) which could limit the cost of nutrient supplementation for fermentation media and; 5) high moisture content. The apple industry waste can be directly used for the production of LA without any additional supplementation of nutrients. In order to increase the LA yield from AP, polysaccharides have to be hydrolyzed, leading to solutions containing high concentrations of sugars and other fermentable compounds.

Gullon et al. [74] conducted a study for L-lactic acid production from AP through sequential hydrolysis and fermentation using *L. rhamnosus* CECT-288. The effects of the cellulase-to-solid ratio (CSR) and the liquor-to-solid ratio (LSR) on the kinetics of glucose and total monosaccharide generation were assessed in this study, and a set of mathematical models were developed to reproduce and predict the hydrolysate composition. During this study, the AP was subjected to enzymatic hydrolysis. Glucose and fructose present as free monosaccharides in the raw material were dissolved at the beginning of the process. Using low cellulase and cellobiose activity (8.5 FPU/g-solid and 8.5 IU/g-solid, respectively) and short reaction times (12 h), 79.1% of total glucan fraction was saccharified, leading to solutions containing up to 43.8 g/l of monosaccharides (glucose, 22.8 g/l; fructose, 14.8 g/l; xylose +mannose+galactose, 2.5 g/l and arabinose+rhamnose, 2.8 g/l), 1.8 g/l of sucrose and 0.9 g L-malic acid/l. It was observed that 93% of uronic acids were solubilized during the enzymatic hydrolysis. The volumetric productivity of monosaccharide production was 3.65 g/l/h, and the monosaccharide/cellulase ratio was 0.06 g/FPU. A quantitative retention of lignin in the residual solid was observed. These enzymatic hydrolyzates were used as substrate for LA production through fermentation with *L. Rhamnosus*. The results indicated the production of 32.5 g L-lactic acid/l. The volumetric productivity and the product yield were 5.41 g/l h

Table 3
Bioproduction of organic acids using AP and APS as substrates.

Organic acid	Substrate	Microorganism	Process	References
Citric acid	AP	*A. niger* NRRL 567, NRRL 2001	SSF-Erlenmeyer flask, tray and rotating drum type solid-state bioreactor	[39,40,48,53]
	AP	*A. niger* van. Tieghem MTCC 281	SSF-Erlenmeyer flask	[107]
	AP	*A. niger* NRRL 567	SSF-Erlenmeyer flask	[80,85]
	AP	*A. niger* BC1	SSF-multilayer packed bed bioreactor	[153]
	AP hydrolysate	*A. niger* PTCC 5010	SmF-Erlenmeyer flask	[10]
	AP	*Aspergillus niger*	Surface culture method	[85]
	APS	*A. niger* NRRL 567	SmF	[39,44,45,49]
Lactic acid	AP	*Lactobacillus rhamnosus* CECT-288	SmF	[73,74,75]

and 0.88 g/g, respectively. Mass balances showed that 46.5 kg of LA can be produced from 100 kg of dry AP by sequential hydrolysis and fermentation. Furthermore, 13.4 kg of oligosaccharides (which can be used as ingredients for functional foods) and 8.2 kg of microbial biomass (which can be used as probiotic) were produced simultaneously.

3.2. Enzymes

The bioprocessing of AP for the production of various enzymes is provided in Table 4. Conventionally, the bioproduction of enzymes is very expensive and raw material translates into 40–60% of the production cost [78]. Literature is replete with use of many agro-industrial wastes, including AP as raw materials for the production of diverse VAPs, such as enzymes. Among various microorganisms, filamentous fungi are widely employed in SSF due to their ability to grow efficiently on complex solid substrates and production of wide range of extracellular hydrolytic enzymes. SSF technology results in an enzyme preparation, which is more concentrated and, hence, best suited for biomass conversion applications [50].

3.2.1. Cellulases and hemicellulases

Cellulases hydrolyze the β-1,4-glucosidic linkages of cellulose to produce glucose, cellobiose and cello-oligosaccharides as primary products. Cellulases are currently the third largest industrial enzyme worldwide, by dollar volume, mainly due to their wide applications in bioethanol production, cotton processing, paper recycling, juice extraction, as detergent enzymes and animal feed additives. Recently, cellulases are gaining interest due to their non-specific activity for hydrolysis of CTS to low molecular weight CTS (LMWCs) and chitooligosaccharides which find potential applications in biomedicine, pharmaceuticals food and agriculture sectors [51]. In fact, cellulases may become the largest volume industrial enzyme, in case ethanol produced from abundant lignocellulosic biomass through enzymatic route becomes a foremost transportation fuel.

Cellulase is the most extensively studied multiple enzyme complex comprised of: (1) endoglucanase or endocellulase (endo-1,4-β-D-glucanase, EC3.2.1.4) which breaks internal bonds to disrupt the crystalline structure of cellulose and expose individual cellulose polysaccharides chains; (2) exoglucanase or exocellulase (1,4-β-D-glucan-cellobiohydrolase, EC3.2.1.91) which cleaves 2–4 units from the ends of the exposed chains; and (3) cellobiase or β-glucosidase (β-D-glucoside glucanohydrolase, EC 3.2.1.21) which hydrolyzes the endocellulase product, cellobiose, into monomer units, glucose, thereby completing the hydrolysis. The absence or low activity of BGL results in accumulation of cellobiose which inhibits the action of other cellulases. On commercial scale, BGL is commonly produced by known hyper-cellulase producing Aspergillus and Trichoderma strains. The cellulase systems of *Trichoderma reesei* are generally found to be deficient in BGL activity. For efficient activities of cellulase enzyme complex, Trichoderma strains are usually co-cultured with Aspergillus strains which are known to produce high titers of BGL [41]. The complete cellulase system comprising CBH, EG and BGL components thus acts synergistically to convert crystalline cellulose to glucose. Hence, for complete hydrolysis of lignocellulosic biomass, an efficient enzyme cocktail having balanced cellulase (containing both endo- and exoglucanase and BGL) and hemicellulase (xylanases) activities are required. At the same time, high titer of cellulase activities is required for feasible production of bioethanol. Xylanases also act along with cellulases complex for the efficient conversion of lignocellulosic substances having appreciable amounts of xylan (hemicellulose) residues to readily available sugars for feasible bioethanol production.

Xylanases are the group of enzymes that are involved in the hydrolysis of xylans and arabinoxylan polymers. These enzymes include endo-1,4-β-xylanase, β-xylosidase, a-arabinofuranosidase and acetylxylan esterase. Xylanases hydrolyze 1,4-β- D-xylosidic

Table 4
Bioprocessing of AP waste for enzyme bioproduction.

Application	Type of fermentation	Microorganisms	References
Cellulase			
β-glucosidase (BGL)	SSF-Erlenmeyer flasks and plastic trays	*A. niger* NRRL 567	[41,50]
	SSF-Erlenmeyer flasks	*M. phaseolina*	[103]
Exoglucanase (FPU)	SSF-Erlenmeyer flasks and plastic trays	*A. niger* NRRL 567	[42,47,50]
	SSF-Erlenmeyer flasks	*M. phaseolina*	[103]
	SSF-Erlenmeyer flasks	*Candida utilis*	[175]
Endoglucanase (CMCase)	SSF-Erlenmeyer flasks and plastic trays	*A. niger* NRRL 567	[42,47,50]
	SSF-Erlenmeyer flasks	*M. phaseolina*	[103]
	SSF-Erlenmeyer flasks	*Trichoderma* sp. GIM 3.0010	[163]
Hemicellulase			
Xylanase	SSF-Erlenmeyer flasks and plastic trays	*A. niger* NRRL 567	[43,50]
		M. phaseolina	[103]
Xylanase	SSF	*Candida utilis*	[175]
Ligninolytic			
MnP	SSF	*Candida utilis*	[175]
Lignin peroxidase	SSF-Erlenmeyer flasks	*P. chrysosporium* ATCC 24275	[68,69]
MnP			
Laccase			
Amylase	SSF-Erlenmeyer flasks	*M. phaseolina*	[103]
Chitinase	SSF-Erlenmeyer flasks and plastic trays	*A. niger* NRRL 567	[42]
Chitosonase	SSF-Erlenmeyer flasks and plastic trays	*A. niger* NRRL 567	[42]
Pectinase enzymes			
Pectinase	Petri dishes	*Aspergillus foetidus*	[86,87]
Pectinase	SSF	*Candida utilis*	[175]
Pectin methylesterase (PME) (pectin esterase)	SSF-trays & 15 l horizontal solid state stirred tank reactor (AP+soya flour, wheat bran and simple mineral salts)	*A. niger* A 163 (MZKIBK)	[12]
	SSF-Erlenmeyer flasks	*A. niger*	[99,100]
Pectolytic	SSF-Erlenmeyer flasks	*A. niger MK-15*	[122]
Polygalactouronase	SSF-Erlenmeyer flasks	*Aspergillus niger* GHRM5	[156]

Abbreviations: BGL, β-glucosidase; SmF, submerged fermentation; SSF, solid-state fermentation.

linkages in xylan to produce xylo-oligosaccharide. Xylan is the major hemicellulose component and approximately accounts for 20–35% of plant cell wall dry weights [92]. The heterogeneity of xylan led to a diversity of xylan-degrading enzymes [56]. Xylanases play a vital role in the context of green energy for the efficient conversion of lignocellulosic substances having appreciable amounts of xylan (hemicellulose) residues to readily available sugars for feasible production of biofuels and other value added products. Xylanases are critical for the feasibility of the bioethanol production process using lignocellulosic wastes.

High cost of cellulase enzymes is a major barrier in various biotechnological processes using lignocellulosic biomass. According to an estimate, cellulases procured from commercial sources contribute 22.5–43.4% to the total cost of cellulosic ethanol production [181]. The cost economics of cellulases and other industrial enzymes can be brought down by multifaceted strategies, such as by using low cost agro-industrial wastes as substrates, cost effective fermentation techniques, such as koji fermentation, supplementation of inducers, careful optimization of vital process parameters for higher enzyme production and simple purification techniques [38,50]. Koji fermentation has recently gained attention for the production of microbial enzymes due to various advantages over traditional SmF [20,132,38,41–43]. Castilho et al. [24] compared the production of lipases using SmF and SSF. The authors reported that the unitary production cost of enzymes produced through SSF is about 3-folds lower as compared to SmF. In this context, the cheap agro-industrial wastes can be used as ideal raw materials for the production of enzymes. Among various microorganisms, filamentous fungi are widely employed in SSF due to their ability to grow efficiently on complex solid substrates and production of wide range of extracellular hydrolytic enzymes. Among the filamentous fungi, *A. niger* is widely used for cellulases production due to higher levels than other fungi, bacteria and yeasts.

Recently, cellulases and xylanases production was carried out through SSF in Erlenmeyer flasks and plastic trays using AP as the substrate. In an attempt to increase enzyme production, the authors used lactoserum as a moistening medium and crude inducer for fungal cellulases and hemicellulase induction [50]. The utilization of AP supplemented with 1% (w/w) rice husk and without any pretreatment for BGL bioproduction using *A. niger* NRRL 567 was demonstrated [41]. The results established that no expensive media was required and, furthermore, the use of inexpensive agro-industrial wastes will have important economic and environmental advantages. The cheap and feasible BGL bioproduction using low cost wastes could be an attractive alternative source of BGL which is an important component of cellulase complex for efficient hydrolysis of lignocellulosic biomass. Dhillon et al. [43] studied the xylanase production by *A. niger* NRRL-567 in SSF using 2^4 factorial design and response surface methodology. The parameters optimized were the initial moisture level and concentration of inducers [veratryl alcohol, copper sulfate and lactose]. Initial moisture level and LAC were found to be the most important variable for xylanase production. The highest xylanase production observed was 3598 ± 65 IU/g (48 h) and 3952 ± 78 IU/g dry substrate (72 h) in Erlenmeyer flasks and plastic trays under optimal conditions using initial moisture of 85% (v/w), pH 5.0 and inducers veratryl alcohol (2 mM/kg), lactose 2% (w/w), and copper sulfate (1.5 mM/kg). More recently, the inherent potential of the phytopathogenic fungus, *Macrophomina phaseolina,* was evaluated for the production of various hydrolytic enzymes, such as cellulase, hemicellulase and amylase using AP supplemented with 1% (w/w) rice husk [103]. Among the two different isolates, microsclerotial (MphP) and mycelial (MphM), MphP was observed as a potential source of different hydrolytic enzymes as compared to MphM. This study reported for the first time the potential of carbohydrate degrading enzyme bioproduction by different isolates of *M. phaseolina*. The enzyme production studies by *Macrophomina* may lead to new directions with respect to modulating effective plant protection strategies as well as higher production of industrially relevant enzymes, using abundant but untapped lignocellulosic biomass. This study may serve to increase the understanding of different substrates that affect the production and role of fungal cellulases in phyto-pathogenicity. These enzymes can be employed directly or complemented to the industrial strains, such as *T. reesei,* via mutagenesis or DNA recombinant techniques with the purpose of enhanced enzymatic activity.

In another study, the potential of AP for production of exo- and endoglucanase by *A. niger* NRRL 567 was demonstrated [42]. Interestingly, the crude enzyme preparation also showed chitinolytic and chitosanolytic enzyme activities which were comparably higher than in the organisms specifically used for the production of these enzymes. The study indicated that the *A. niger* strains can be cultured for higher production of chitinolytic and chitosanolytic enzymes which find promising applications in various sectors. Generally, chitinolytic and chitosanolytic enzymes are used for depolymerization of chitin and CTS to make LMWCs and chitooligomers [34]. However, the applications of chitosanases are hindered by their high cost and lower availability. Various other enzymes, such as cellulases, lipases, lysozyme, papain and pectin lyase, have been utilized for hydrolysis of CTS to CTS preparations with different molecular masses [152,111,112,183]. Cellulase–chitosanase dual activity has been reported for *Streptomyces griseus* MUT6037, *Myxobacter* A-L1, and *Bacillus megaterium* P1 [133,137,82]. Recently, cellulases have also been finding potential applications owing to their multifunctional activities, such as nonspecific hydrolysis of chitin and CTS to chitooligomers and LMWCs [51]. These products find innumerable applications in various fields, such as biomedical, pharmaceutical, biotechnological, food, and agricultural sectors [183]. The cellulases are preferred over specific chitinases and chitosanases because of their low production cost and for comparable, or even superior CTS hydrolysis rates [190,112,88].

Cellulases and hemicellulases produced by koji fermentation can find promising applications for saccharification of lignocellulosic biomass to high biotechnological value sugar syrups. These sugar syrups can be used for the bioproduction of biofuels and various other VAPs. Since, arrays of enzyme activities are required for the complete hydrolysis of cellulose, crude cellulase preparations are considered superior over the purified enzymes for industrial use. Moreover, SSF results in concentrated enzyme preparation. In fact, the efficiency of lignocellulosic hydrolysis can be dramatically increased if the crude cellulase from the fermentor is directly used for industrial purpose.

3.2.2. Ligninolytic enzymes

It is now well acknowledged that SSF is the most economical process for ligninolytic enzyme production by fungi [89]. Over the past decade, various white rot fungi, such as *Trametes* and *Phanerochaete chrysosporium,* by virtue of production of ligninolytic enzymes have been studied for their ability to degrade recalcitrant environmental pollutants, such as sulfonphthalein, DDT, lindane, dioxin and benzopyrene. Among white rot fungi, the basidiomycete, *P. chrysosporium,* has become the most widely used fungus due to its ability to secrete extracellular ligninolytic enzymes fast growth and easy handling during culturing techniques. This microorganism has now become a model for many studies on bioremediation of pollutants, such as polynuclear aromatic hydrocarbons. Besides this, *P. chrysosporium* is also used for the production of several extracellular ligninolytic enzymes, such as manganese peroxidase (MnP), lignin peroxidase (LiP) and laccase, that are capable of degrading lignin. Recently,

Gassara et al. [68] studied the ligninolytic enzyme [LiP, MnP and laccase] secretion ability of *P. chrysosporium* by using AP and other agro-industrial wastes supplemented with inducers for higher secretion. Results indicated that AP resulted in higher enzyme production by *P. chrysosporium* BKM-F-1767 as compared to other agro-industrial wastes, such as brewery waste, paper and pulp industry sludge and fishery waste. Higher laccase and peroxidase (LiP and MnP) activities were achieved with AP, possibly due to insoluble carbohydrates, such as cellulose, hemicellulose, and lignin content of the AP, which can stimulate laccase and peroxidase (LiP and MnP) production by *P. chrysosporium.* In addition, high concentrations of carbon (62% w/dw) and nitrogen (6.8 g/kg) present in dry AP can act as important parameters to enhance enzyme activities. Therefore, there is an enormous potential of AP as a low cost substrate for economical ligninolytic enzyme bioproduction. In another study conducted by Gassara et al. [69], LiP, MnP and laccase production by *P. chrysosporium* using AP waste was optimized through response surface methodology. The effect of moisture, copper sulfate and veratryl alcohol (VA) concentrations on enzyme production was studied. The results indicated that moisture and VA had significant positive effect on MnP and LiP production and the viability of *P. chrysosporium* ($p<0.05$) whereas moisture and copper sulfate were observed to had a significant positive ($p<0.05$) effect on laccase production. Higher values of MnP, LiP and viability of *P. chrysosporium* on AP (1287.5 U MnP/gds (units/g dry substrate), 305 U LiP/gds, and 10.38 Log 10 viability) were obtained with 80% moisture, 3 mmol/kg VA, and 0.5 mmol/kg copper. Higher production of laccase using AP (789 U/gds) was obtained with 80% moisture, 3 mmol/kg VA, and 1.5 mmol/kg copper sulfate. Villas-Bôas et al. [175] utilized *Candida utilis* to produce ligninolytic enzymes (pectinase, manganese-dependent peroxidase, cellulase and xylanase) by using AP under SSF conditions. The study reported low yield of manganese-dependent peroxidase activity at 19.1 U/ml. The ligninolytic enzymes are gaining interest due to their environmental applications for degradation of various recalcitrant pollutants. For economical production of these enzymes, apple industry and other agro-industrial wastes can be used through SSF.

3.2.3. Amylases

Amylases are one of the most important industrial enzymes worldwide. They form an enzyme complex comprising enzymes that act synergistically to break down starch to glucose. The starch polysaccharides amylose (15–25%) is composed of linear α-1,4-linked glucose units, and amylopectin (75–85%) is a α-D-glucose-based homo-polymer with linear chains and α-1,6-linked branches. Thus, amylolytic complexes are formed by three major groups of enzymes: (1) endoamylases; (2) exoamylases; and (3) debranching enzymes. Endoamylases (also known as liquefying or depolymerizing enzymes) are composed mainly of α-amylases (EC 3.2.1.1) and release oligosaccharides of different chain lengths by randomly hydrolyzing the internal α-1,4 linkages. Exoamylases, also called saccharifying enzymes, composed generally of glucoamylases (EC 3.2.1.3 glucan 1,4-α-glucosidase), release glucose as the main product, by cleaving terminal α-1,4 bonds. The debranching enzymes, e.g. pullulanase (EC 3.2.1.41), act mainly on α-1,6 linkages of amylopectin. Amylases play a pivotal role in the bioethanol production from corn and other starchy substrates. Besides, starch industry approximately accounts for up to 15–20% of the total industrial consumption of enzymes. Among these glucoamylase comprises a major component besides α-amylase and glucose isomerase [106].

The ability of the plant pathogenic fungus, *M. phaseolina,* was evaluated for amylase production using AP through SSF in Erlenmeyer flasks by Kaur et al. [103]. Higher amylase activity of 3309.45 ± 29.22 IU/gds was recorded with optimum conditions having a temperature of 35 °C using MphP at 70% (v/w) initial moisture level after 120 h incubation time. Considering the importance of amylases in food and other industries, apple processing wastes can be used for the economical production of these enzymes to fulfill the needs of industries for the development of cost-effective processes.

3.2.4. Pectinases

Pectinases represent a heterogeneous group of enzymes that catalyze the hydrolysis of pectins, which are the structural polysaccharides present in plant cells and are responsible for maintaining the plant tissues integrity. Pectinase enzymes comprise (1) pectin methylesterase (pectin esterase); (2) depolymerizing enzyme polygalacturonase; and (3) pectin lyases. Pectin esterase hydrolyzes the pectin to methanol and polygalacturonic acid [21] and the enzyme polygalacturonase further hydrolyzes the polygalacturonic acid into monogalacturonic acid by breaking the glycosidic linkage [144]. Pectinases find extensive applications in fruit processing industries including clarification of fruit juices and wines, extraction of fruit juice, in the manufacturing of pectin free starch, to improve oil extraction, curing of coffee, to remove the peel from citrus fruit, to increase the firmness of several fruits and to degum fibers [62,100].

Owing to the presence of pectin in the AP, it is an ideal substrate for the induction of pectin esterase. Several microorganisms are known to produce pectinase enzymes, such as *Aspergillus* and *Trichoderma* sp., *Fusarium moniliforme* and *Rhizocotonia solani*. Joshi et al. [99] evaluated pectin methyl esterase (PME) production using AP in SSF and SmF. The results of the study indicated that SSF resulted in enzyme activity of 6.75 U/g that was about 2.3 times higher at a dilution level of 1:3 compared to SmF. SSF is considered more suitable than SmF for the growth of filamentous fungi [134]. Higher productivities of endo- and exo-pectinase and pectin lyase were obtained with SSF than SmF, using *A. niger* [1]. Besides supplying the nutrients to the microbial cultures growing on it, solid substrates also serve as an anchorage for the cells [136]. Moreover, low moisture content of fermenting medium in SSF might prevent the bacterial contamination [155,102]. Earlier studies reported that the regulatory mechanism of pectin methylesterase production by *A. niger* in SSF and SmF is different. In SSF, pectinase synthesis is least affected by the catabolic repression than SmF [157].

Recently, Joshi et al. [100] carried out the purification and characterization studies of pectinase produced from AP by *A. niger* and evaluation of its applications in fruit juice extraction and clarification. The results demonstrated that the enzyme could be successfully employed for extraction and clarification with improvement in sensory qualities and without altering its physico-chemical characteristics of plum, peach, pear and apricot juices. Various studies have demonstrated the potential of AP for pectinase production [86,87].

3.2.5. Other enzymes

Recently, Siva Kiran et al. [156] carried out statistical optimization of medium composition for producing endo-polygalacturonase by mutated strains of *A. niger* GHRM5 using AP waste through SSF technique. The Plackett–Burman design was used to search for the main factors. To study the mutual interactions between variables and find the optimum medium, the Doehlert design was performed to investigate the effect of the medium components, AP, glucose and urea. Urea was found to be the most significant factor ($p<0.05$) among all variables. Using the optimized medium, the maximal activity of endo-polygalacturonase production was found to be 4.266 U. The Plackett–Burman design coupled with the Doehlert

design was proved to be a potent tool in optimizing medium composition for producing endo-polygalacturonase.

Studies were carried out by Villas-Boas et al. [175] to improve the nutritional value of AP as ruminant feed which has low digestibility due to high lignin/cellulose ratio. After SSF of AP by *C. utilis* CCT 3469, degradation of cellulose, pectin and lignin fragments was studied. Lignocellulolytic enzyme production by *C. utilis* was investigated and results showed that high activity for pectinase (239 U/ml) as well as a significant MnP (19.1 U/ml) activity was achieved. However, low cellulase (3.0 U/ml) and xylanase (1.2 U/ml) activities were also observed suggesting that *C. utilis* may have lignocellulose degradation ability.

3.3. Natural antioxidants

Various polyphenolic compounds have been extracted from AP as listed in Table 5. Due to health and environmental awareness, sustainable food production and value addition of agro-industrial wastes are the principal issues in the agro and food processing industry. Apple and apple products are one of the major fruit and fruit products consumed all over the world. AP is an excellent source of natural antioxidants, such as catechins, procyanidins, caffeic acid, phloridzin, phloretin glycosides, quercetin glycosides, and chlorogenic acid, among others. AP, including seeds, contains polyphenolics with the strong antioxidant activity of quercetin glycosides, phloridzin and its oxidative products [113,114,148,149,77,26].

Balanced diet rich in fruits and vegetables is gaining more importance due to their significant role in reducing the risk of certain types of cancer, cardiovascular and other chronic diseases [101,119,161]. Fruits and vegetables are a rich source of many antioxidant compounds, such as phenolic compounds, carotenoids, anthocyanins and tocopherols among others [125]. Apple is an important resource having bioavailable polyphenols, such as flavonols, monomeric and oligomeric flavonols, dihydrochalcones, and anthocyanidins, among others [61]. The most plentiful polyphenols occurring in apples are chlorogenic acid, phloretin glucosides and quercetin glucosides ([180]). Although present in relatively small quantities, other polyphenolic compounds, such as catechins and procyanidins, have also been detected [65].

Depending upon different varieties and various parts of apples, the polyphenolic compound contents vary largely. Apple peels contain a higher concentration of phenolic compounds than the whole apple [179]. In recent times, owing to the increasing interest in new natural sources of antioxidant products, AP has been researched as a promising source of bioactive polyphenols [26]. These bioactive polyphenols have many potential applications in food, pharmaceutical and cosmetic industry by virtue of their antioxidant and antimicrobial activities.

Eberhardt et al. [57] studied the anti-tumor activity of polyphenols from apple extracts which have been shown to inhibit in vitro tumour-cell proliferation. In another study conducted by Lu and Foo [114], the antioxidant properties of AP polyphenols were evaluated using a β-carotene/linoleic acid system, free radical scavenging activity using DPPH (1,1-diphenyl-2-picryl-hydrazyl) and superoxide anion radical scavenging activities by a cellular xanthine/xanthine oxidase system as a superoxide source. Epicatechin and quercetin 3-glycosides from pomace showed the highest activity, while phloridzin exhibited moderate activities in comparison to vitamins C and E. Except for phloridzin (0.60 EC50), all the apple polyphenols exhibited good DPPH-scavenging properties, which were significantly (2–3 times) higher than vitamin C (0.35 EC50) and E (0.30 EC50). EC50 is the amount of antioxidant activity necessary to decrease the initial DPPH concentration by 50%. As evident from the results, AP polyphenols were found to be effective superoxide scavengers, in comparison to vitamins C and E. Procyanidins were observed to be superior to quercetin 3-glycosides, chlorogenic acid, 3-hydroxyphloridzin and phloridzin. These studies indicated that the polyphenols responsible for the antioxidant activity in apple were also present in the AP and can be extracted for food fortification or nutraceutical product development. AP can therefore become an inexpensive and readily available source of natural antioxidants.

Although the potential of AP as a source of polyphenols seems clear, there is scanty information on potential strategies for the liberation and recovery of these vital compounds. Mostly, the polyphenolic compounds are present in conjugated form with one or more sugar residues linked to hydroxyl groups as well as with other compounds, such as organic acids, amines, and lipids [19]. The polyphenolics in conjugated form as glycosides have reduced functionality as good quality antioxidants since the resonance stabilization of free radicals mainly depends on the availability of free hydroxyl groups on the phenolic rings [170]. The lowered antioxidant activity in turn has a direct effect on the health

Table 5
Natural antioxidants present in apple industry waste.

Total phenolic compounds present in apple industry waste		
Apigenin[a,b]	Kaempferol-*O*-glucoside[b]	Quercetin 3-diglucoside [b]
Catechin 1[c]	Luteolin[a,b]	Quercetin-*O*-pento-hexoside[a,b]
Chrysoeriol[a,b]	Luteolin-7-*O*-glucoside[a,b]	Quercetin 3-arabinofuranoside[b]
Cyanidin 3-glucoside[b]	Luteolin-7-*O*-galactoside[a,b]	Quercetin 3-rhamnoside[b]
Epicatechin[b]	Naringenin[a,b]	Quercetin 3-*O*-rhamnoside[c]
p-Coumaric acid[d]	Naringenin-7-*O*-rutinoside[a,b]	Quercetin-*O*-pentoside[b]
p-Coumaroylquinic acid[d]	Naringenin-*O*-hexoside[a,b]	Quercetin 3-rutinoside [b]
Caffeic acid-*O*-glucoside [b]	Naringenin-*O*-hexoside[a,b]	Quercetin 3-galactoside [d]
Chlorogenic acid [d]	Naringenin-*O*-glucuronide[a,b]	Quercetin 3-glucoside [b]
Caffeoylquinic acid[b]	Naringenin-7-*O*-neohesperidoside[a,b]	Quercetin 3-xylanoside [b]
Cyanidin 3-glucoside[b]	Naringenin-7-*O*-glucoside[a,b]	Quercetin-*O*-hexoside [b]
Dicaffeoylquinic acid [b]	Procyanidin B2 [d]	Quercetin 3-arabinopyranoside[b]
Epicatechin[c]	Phloridzin [d]	Rhamnetin[a,b]
Eriodictyol-hexoside[a,b]	Phloretin [d]	Rhamnetin 3-glucoside[a,b]
Eriodictyol[a,b]	Phloretin xyloglucoside [d]	Sinapic acid-*O*-glucoside[b]
Ferulic acid [b]	Protocatechuic acid[a,b]	Salicylic acid[a,b]
Hesperidin-*O*-pentoside[a,b]	Quercetin [b]	
3-Hydroxyphloridzin [b]	Quercetin 3-*O*-glucoside[c]	

[a] Reported for the first time in apple pomace.
[b] Present in apple pomace fraction.
[c] Present in apple seed.
[d] Present in both apple pomace fraction and seed.

functionality when these compounds enter into the body through food or nutraceuticals, and may have to depend on the probiotic status of the digestive system [170]. Therefore, the release of free phenolics can improve the health functionality of these phytochemicals. Various ligninolytic and carbohydrate metabolizing enzymes are synthesized by fungi during fermentation of lignocellulosic wastes. These enzymes can hydrolyze the phenolic glycosides and can release the free aglycones, potentially having high antioxidant activity, making them very valuable for applications in food and beverage industries [170].

The carbohydrate-cleaving enzyme, such as BGL (β-D-glucoside glucohydrolase), catalyzes the hydrolysis of glycosidic linkages in alkyl or aryl β-glucosides as well as glucosides containing only carbohydrate residues [124,115]. The enzyme is capable of hydrolyzing phenolic glycosides and releasing extractable free aglycones potentially having high antioxidant activity, therefore making them very useful for applications in food and beverage industries [169]. Cellulose-degrading cultures of the white rot basidiomycete, *P. chrysosporium*, were reported to secrete three different β-glucosidases depending on the carbon source: (1) extracellular; (2) intracellular; and (3) cell wall bound [110]. However, a study conducted by Duhalt et al. [55] reported that lignifying and tannin-forming peroxidases and fungal enzymes can cause polymerization, depolymerization, and lignification of the released polyphenolics which in turn reduces the nutraceutical property of the products.

Recently, a study was carried out to understand the changes and liberation of phenolic compounds and improvement in antioxidant activity during SSF using AP with *P. chrysosporium* [5]. The results indicated that SSF of AP using *P. chrysosporium* mobilized the polyphenolic compounds and improved the nutraceutical properties. The polyphenol content in acetone extract increased significantly ($p < 0.05$) from 4.6 to 16.12 mg gallic acid equivalents per gram dry weight (GAE/g dw) during SSF. The effect of various solvents, temperature, time and detergents/non-ionic surfactants was also examined for the extraction of polyphenolics by ultrasonication and microwave-assisted extraction methods. The polyphenol content of the extracts was found to be in the range of 5.78–16.12 mg GAE/g dw of samples, depending on the solvent, extraction time and temperature.

A study was conducted by Ajila et al. [6] to evaluate the effect of different fermentation techniques, such as flask, tray, and fermentor, for improving the antioxidant properties of polyphenolics and understand the changes and mobilization in AP using *P. chrysosporium*. Various activities, such as BGL, ligninolytic enzymes and polyphenolic-linked antioxidant activity during SSF, were also studied. The results indicated that during the course of SSF, there was an increase in the extractable polyphenolic content (15.53–29.28 mg of GAE/g dw) on the 7th day followed by a decline in the polyphenol content. Antioxidant activity was measured by DPPH radical inhibition system. The increase in activity of about 35% was directly proportional to polyphenolic content over the course of SSF. Both polyphenolics and antioxidant capacity correlated with the increase in the BGL activity and showed that the enzyme played an important role in the release of polyphenolic aglycones from AP and therefore increased the antioxidant capacity. In addition, the study also demonstrated that ligninolytic enzymes showed a direct correlation with the mobilization and polymerization of polyphenolic content during the SSF.

3.4. Dietary fibers

Dietary fibers are the plant cell wall compounds (non-starch polysaccharides), which are resistant to hydrolysis by digestive enzymes in humans. They are intrinsic and intact in plants and mainly comprised of cellulose, hemicelluloses, pectic substances and lignins. Diets with insufficient dietary fiber content are generally associated with various abnormalities, such as constipation, diverticulosis, cardiovascular disease and cancer [167]. Dietary fibers impart a number of protective effects on cardiovascular diseases, colorectal cancer, obesity and diabetes [145,60]. These dietary fibers are known to bind excess hydrochloric acid, cholesterol and gastric juices, increase the fecal bulk and stimulate intestinal peristalsis [91,127]. The fruit and vegetable-based fibers are known to have improved nutritional value than cereal formulations, due to the presence of various associated active compounds, such as flavonoids, carotenoids, and higher fiber content in balanced composition (soluble/insoluble dietary fiber ratio, water- and fat-holding capacities, lower energy value and phytic acid content).

AP contains a significant amount of non-starch polysaccharides (35–60% dietary fiber), with a high amount of insoluble fiber (36.5%) as well as soluble fiber (14.6%) [30,67,177,162]. The main constituents of AP dietary fibers are pectins (5.50–11.70%), cellulose (7.20–43.60%), hemicelluloses (4.26–24.40%), lignins (15.30–23.50%) and gums. Cellulose, pectin and lignin are water insoluble, whereas galacturonic acid and hemicelluloses are water soluble. A number of fiber enriched bakery products were prepared by supplementing dietary fibers and dried AP powder [30,116,162]. Various authors have advocated the addition of apple fibers (up to 4%) for food enrichment without compromising product quality and acceptance by consumers.

3.5. Aroma compounds

Flavors are important components of food industry and comprise over a quarter of the world market for food additives. Most of the flavoring compounds are produced via chemical synthesis or by extraction from natural materials. However, recent market surveys have shown that consumers prefer foodstuff that can be synthesized naturally due to health awareness. Plants are major sources of essential oils and flavors. However, their utilization depends on natural factors that are difficult to control, such as weather conditions and plant diseases. An alternative way for flavor synthesis is via microbial biosynthesis or bioconversion [90]. Various microorganisms, such as bacteria and fungi, are currently known for their capability to synthesize different aroma compounds. A study showed that SmF rendered low productivity of aroma compounds [184], which hindered their industrial application. Ferron et al. [64] reviewed the prospects of microbial production of food flavors and suggested the suitability of SSF processes for production of aroma compounds. Bramorski et al. [18] demonstrated the potential of SSF for fruity aroma production by *Ceratocystis fimbriata* using several agro-industrial wastes, such as AP, amaranth, cassava bagasse and soybean. The results indicated that the media containing AP, cassava bagasse or soybean produced a strong fruity aroma.

3.6. Biofuels

The uncertainty of availability of nonrenewable oil resources to meet the fuel needs of growing population and concerns about the global climate change has led to a search for alternative fuels. Due to abundant availability and renewable nature, lignocellulosic wastes especially agro- industrial wastes appears to have immense commercial potential for bioethanol production. The worldwide drive to reduce the competition between crop usage for food and non-food applications has prompted massive research efforts to utilize the abundant but untapped lignocellulosic wastes. The importance of lignocellulosic ethanol (second generation biofuels) stems from the possibility to use inexpensive feedstock, avoid

direct and indirect competition with human food and animal feed and reduce the environmental risks i.e. soil degradation, and water and air pollution which are associated to first generation biofuels.

Feasibility of various lignocellulosic materials for sustainable and economical production of bioethanol has been evaluated throughout the world. Lignocellulosic ethanol is expected to be commercialized during the next decade as renewable energy for transport. Various studies have demonstrated the potential of AP as a substrate for bioethanol production [79,128,129,94,147,29]. SSF has been carried out for the production of ethanol from AP using consortia of cultures including *S. cerevisiae* MTCC 173 (ethanol producer), *A. foetidus* MTCC 117 (pectinase producer) and *F. oxysporum* MTCC 1755 (cellulase producer). This fermentation process yielded 16.09% (v/w of AP) ethanol from fermented AP with a residual sugar of 0.15% (w/w of AP). The study demonstrated the potential of AP for the concomitant production of ethanol as an efficient method for alleviating waste disposal. The economical potential of SSF remains to be assessed. During fermentation with pure culture of *S. cerevisiae* MTCC 173 (1% inoculum) 8.44% (v/w) ethanol was recovered after 72h of incubation at 30° C. However, with the use of co-cultures i.e. *S. cerevisiae* MTCC 173, *A. Foetidus* MTCC 151, *F. oxysporum* MTCC 1755 the ethanol yield increased to 16.09% (v/w) and sugar concentration further decreased to 0.15% after 72 h incubation at 30 °C [29]. Recently, Borah and Mishra [17] carried out ethanol production using AP and rotten banana as a substrate by treating it with distilled water, small amount of sucrose and *Saccharomyces cerevisiae* (collected from "FRI, Dehradun", India). After 36 h of fermentation process an ethanol yield of 38% was achieved.

In context of green energy or development of biorefinery approach-based processes, the sustainable utilization of nutrient-rich agro-industrial wastes is a key step. The biological transformation of these wastes to high value products relies on a variety of enzymes, such as cellulases and hemicellulases. Cellulases refer to a family of enzymes which act in synergism to hydrolyze cellulose polymer. Cellulase is widely produced by fungi and other microorganisms. For efficient hydrolysis of lignocellulosic biomass, a complete enzyme cocktail containing balanced cellulase (containing both endo- and exoglucanase) and hemicellulase activities is sought. At the same time, high titer of cellulase activities is required for feasible production of bioethanol. It should also be noted that cellulase preparations generally contain other enzymatic activities besides cellulase, and these may affect the properties of the preparations.

Edwards and Doran-Peterson [58] presented a detailed review regarding the efficient utilization of pectin rich waste, and AP as a potential feedstock for bioethanol production. Pectin-rich wastes have markedly less proportion of lignin as compared to lignocellulosic biomass, such as agricultural wastes. This is important as lignin interferes with the enzymatic hydrolysis of cellulose and hemicellulose [27,11,76]. Moreover, lignin is not fermentable into ethanol. The lignin bonds with carbohydrates must be broken in order to ferment them to produce ethanol. This often requires costly and harsh physical, chemical and/or biological pretreatments that may degrade lignin and some sugars into inhibitory molecules (e.g. furfurals) rendering the ethanol production non-feasible. Hence, apple industry wastes represent rich substrates for culturing of different microorganisms for processing to various high value products. In the US alone, out of the total AP waste generation (0.4 million tons on dry weight basis), 0.08 million tons of bioethanol can be produced [58].

3.7. Biopolymers

3.7.1. Chitosan

CTS is a copolymeric structure comprised of β-(1-4)-2-acetamido-D-glucose (acetylated unit) and β-(1-4)-2-amino-D-glucosamine (deacetylated unit) residues. The development of applications for CTS has expanded rapidly in recent years. CTS is widely used in food, biomedicine, pharmaceuticals, personal care products, agriculture and environmental sectors [51,52]. CTS is generally obtained from crustacean shell wastes resulting from the industrial processing of seafood, such as shrimp, crabs, squids, and lobsters. However, the extraction method suffers from environmental and other disadvantages. CTS also occurs naturally in some fungi belonging to *Ascomycetes*, *Zygomycetes*, *Basidiomycetes* and *Phycomycetes* [141]. The mycelia of several fungi, such as *Mucor rouxii*, *Absidia glauca*, *A. niger*, *Gongronella butleri*, *Pleurotus sajor-caju*, *Rhizopus oryzae*, *Lentinus edodes*, and *T. reesei*, have been considered as possible sources of chitin and CTS due to their presence in the cell walls [52,104].

Streit et al. [160] examined the production of CTS using submerged cultivation of *G. butleri* CCT4274 using AP as substrate. CTS yield of 1.19 g/l culture medium, including 40 g/l of reducing sugars and 2.5 g/l of sodium nitrate, was produced after a 72 h-incubation period. The integrated process was developed for the sequential extraction of CTS from *A. niger* waste mycelium followed by solid-state and submerged CA fermentation in lab scale fermenters using AP and APS with *A. niger* NRRL 567. Extractable CTS was found to be 6.40% and 5.13%, respectively of dried fungal mycelium resulting from the SSF and SmF (Personal data, communicated). Considering the wide range of applications of this important biopolymer, the waste fungal mycelium resulting from different industrial fermentation processes can be viewed as a promising source for economical extraction of high quality CTS.

3.7.2. Xanthan gum

Xanthan gum is a hetero-polysaccharide industrially produced by the bacterium *Xanthomonas campestris* generally through fermentation of glucose or sucrose. It is the most important microbial polysaccharide from a commercial point of view, with a worldwide production exceeding 30,000 tons per year [35]. Being water soluble, xanthan gum is widely used in several industrial applications, such as food, cosmetic, textile and pharmaceutical due to its rheological properties. Besides, xanthan gum has been used as emulsifiers, stabilizers and texture enhancers in the food industry [131].

The exopolysaccharide production using SSF has been reported by the group of Stredansky and Conti [158] and Stredansky et al. [159]. *X. campestris* strains were cultivated on easily available low cost substrates, such as AP, grape pomace and citrus peels, among others, in order to evaluate their ability to produce the exopolysaccharide xanthan. With most of the substrates including AP, xanthan yields were comparable to those obtained from conventional SmF. Moreover, the products were analyzed by NMR spectroscopy, indicating a composition consistent with that of commercially produced xanthan.

3.8. Nutritional enrichment

3.8.1. Culturing of industrially important microorganisms

Besides being a rich source of carbohydrates, AP also contains dietary fibers, vitamins and other vital nutrients necessary for the growth of microorganisms. It can be used as a potential substrate to harness industrial microorganisms like other wastes. AP, being a low bulk and high value by-product, holds great promise for bioproduction of baker's yeast. Traditionally, molasses is the only substrate used to produce baker's yeast. However, from the last decade or so, its utilization is lacking interest due to improved methods of high sucrose recovery and lower fermentable sugar concentration. Additionally the molasses containing medium requires heavy supplementation with costly growth promoting nutrients, such as minerals and vitamins along with clarification

and pretreatments of molasses. Joshi and Bhushan [98] studied the suitability of AP for the production of baker's yeast, *Saccharomyces cerevisiae*. Among all the substrates, glucose gave higher fermentation efficiency followed by AP extract (APE) in terms of higher cellular yield coefficient and lower ethanol production as compared to jaggery and molasses. However, supplementing the medium with growth stimulators (minerals and vitamins) increased efficiency of baker's yeast except APE, in terms of both respiration and to ferment the available sugar. The results indicated that supplementation of APE is not required for the production of baker's yeast.

AP can be used as potential substrate for the production of edible mushrooms [182,186,187–189]. *Pleurotus ostreatus* can successfully be grown in lignocellulosic substrates resulting in edible mushrooms with high protein content [54]. Isolates of *Pleurotus* can grow at relatively low pH values, about 3.5, with an optimal pH of 7.0, and optimal growth temperature at 25 °C [66], which fits aptly to the physico-chemical characteristic of the AP having pH value $\sim 3.5 \pm 0.1$.

3.8.2. Animal/livestock feed

AP is presently used in low value applications, such as to feed ruminants or simply added to soil as a fertilizer. Various studies demonstrated the utilization of AP as a supplement for animal feed ([95,117,4,14,185]). However, various factors adversely affect the value of AP as a ruminant feed: (1) AP has low digestibility due to high lignin/cellulose ratio; (2) has a high free sugar content, which after ingestion by ruminants and fermentation in the rumen causes animal alcoholaemia, a problem for cattle farmers; and (3) the protein, vitamin and mineral content of AP are adequately low, which contributes to the low nutritional level and therefore low commercial value of this waste biomass. Various strategies can be adopted to increase digestibility of lignocellulosic wastes. Biological treatment is an effective method based upon the decomposition of lignin after the splitting of the cellulose–lignin complex as represented in Fig. 3. The main hindrance of biological transformation of lignocellulosic biomass is to select microorganisms capable of degrading the lignin selectively. Suitable microorganisms should metabolize the lignin efficiently and selectively avoiding cellulose and hemicellulose degradation [176].

Biological transformation using various culture strategies, such as involving single or co-culture microorganisms and sequential consortia, has been carried out [2,130,177]. Amongst the various microorganisms used, the yeast *C. utilis* and the filamentous fungus *Pleurotus ostreatus* are the most commonly studied [143,98,99]. *Candida utilis* (Henneberg), syn. *Pichia jadinii*, has been widely used in the production of single cell protein (SCP) for animal feed [13,3,96,97]. *C. utilis* is considered a safe and high nutritional value SCP source by the food and feed industries. Furthermore, *C. utilis* has been used in the conversion of AP giving rise to animal feed with higher protein levels [13,139,96,97]. The yeast metabolizes most of the natural mono- and disaccharides as well as lignocellulosic components such as pectin, xylan, cellulose and lignin fragments [175]. On the other hand, the white-rot fungus *P. ostreatus* is widely used in the delignification of several substrates owing to secretion of ligninolytic enzymes, therefore improving the digestibility of lignocellulosic materials along with parallel protein enrichment in most cases [2,108].

In general, the cost of animal feed is quite high. AP alone is not a suitable feed as it is deficient in digestible protein making it less attractive from an animal nutrition point of view, [143]. Various authors have devised strategies for protein enrichment of AP through fermentation. Growth of yeast on the AP increases protein and vitamin contents [81]. However, the low level of fermentable sugars limits protein enrichment of the AP by yeasts, as a major portion of the pomace comprises lignocellulosics. Bhalla and Joshi [13] attempted to use a co-culture system (with a mold as the cellulolytic organism and yeast as the fermentative organism) to increase the protein content of the pomace. The results showed that the co-culture increased the protein content of dried and pectin-extracted AP to 20% and 17%, respectively, under SSF conditions.

AP was transformed to animal feed on a large scale by SSF under optimized fermentation conditions using five different yeasts i.e., *Saccharomyces cereutstae, C. utilis, Torula utilis, Schlzosaccharomyces pombe* and *Kloeckera* spp. The compositional analysis of fermented AP with yeasts after ethanol recovery revealed that the dried product was rich in crude proteins, crude fat, vitamin C and minerals. Fermented AP, when mixed with a standard broiler feed in 1:1 ratio, was acceptable to broilers and results were comparable to that of standard feed. The broilers gained weight regularly up to 8 weeks. There was neither mortality nor any abnormalities in liver and kidneys of the broilers. The serum biochemical changes i.e., serum urea, nitrogen level, sugar, total proteins, ALT, AST and AKPase levels, were also found to be within the normal limits. The study concluded that reformulation of broiler feed could make AP as one of the essential components of feed [97]. Zafar et al. [185] conducted a study to replace high cost feed ingredients with low cost fruit by-products. This study has been carried out to evaluate the potential of apple by-products as supplement in poultry feed. The results of the study indicated that AP can be used safely as an energy source in broiler ration replacing maize by 10% (w/w) without any side effects on the broiler production. A higher level than 10% creates a problem of wet litter and depressed feed efficiency perhaps due to higher fiber content. Apple by-products can safely replace maize in livestock rations supplemented with multi-enzymes and are a cost effective yet equally good feed adjunct for broiler chicks. Thus apple industry wastes have the potential to replace some costly feed ingredients.

Tiwari et al. [166] conducted a study to evaluate the effect of feeding AP on milk yield and milk composition in crossbred cows as compared to normal diet. The results of the trials indicated that maize can safely be replaced to the extent of 33% by AP in the dairy ration. Results showed no significant effect in milk production of animal fed with or without AP. There was no significant change in fat (%) of milk. However, solid not fat (SNF) level was comparatively higher in the AP fed group. The protein content of milk was comparable. Apple grown areas where there is abundant availability of such feeding material can be used in the diet of lactating animals for cost effective milk production. In their earlier study, Tiwari et al. [165] showed that inclusion of AP in the dairy ration makes it

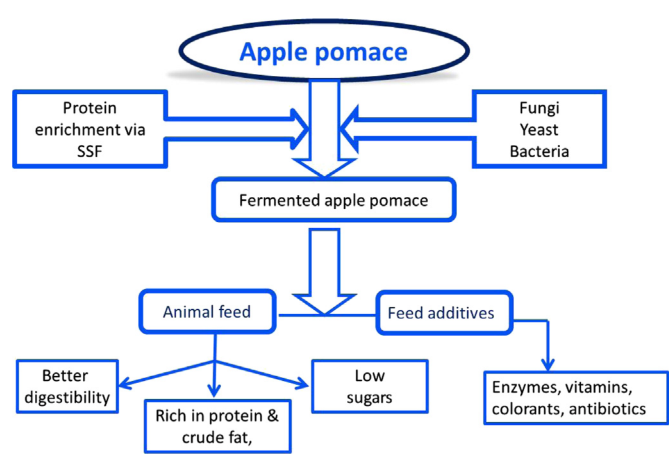


Fig. 3. Diagrammatic representation of biological protein enrichment of apple pomace via solid-state fermentation with microorganisms.

economical and cost effective. Different experiments were conducted to evaluate the nutritional value of AP as a supplement in different ratios (30–60%) with commercial concentrates and rice straw in the diets of goats (*Capra hircus* [4]). The study demonstrated that AP enriched diets had higher dry matter intake, nutrient digestibility and nitrogen retention than diets without AP. The results of this study infer that AP can be supplemented at levels of up to 60% in the diets of goats without any adverse effects [4].

3.8.3. Single cell protein

AP possesses high moisture content, insoluble carbohydrates, such as cellulose, hemicellulose and lignin, reducing sugars, such as glucose, fructose and sucrose, and is low in protein, essential amino acids, salts, and vitamin C [188,190,65,176,95,7,173]. AP is sold to the farmers at a price of approximately US $1.50/ton [173] and is used as an organic compost in crop fields and as animal feed, although its protein content is low. However, when AP is used for animal feed without biological treatment, it can give rise to the phenomenon known as alcoholemia due to the fermentation of AP to produce alcohol in the rumen of the animal causing intoxication [174,175].

Due to its levels of sugars, AP may be an alternative substrate for the production of SCP, which will also result in reduced quantity of fibrous and mineral material. Cultivation of filamentous fungus on AP can transform it into a protein enriched material by the conversion of sugars, nitrogenous material, fibers, and ashes in SCP and could be supplemented to animal feed. Moreover, AP after fermentation process especially by solid-state cultivation of microorganisms can also be used directly i.e. fermented material together with the SCP, eliminating the need for downstream processes [171].

3.8.4. Protein enrichment

Several studies demonstrated the use of AP for the production of protein-enriched feeds [13,175,36,7,171]. Bhalla and Joshi [13] employed the fungi *Trichoderma viride* and *A. niger* and the yeasts *S. cerevisiae* and *C. utilis* combined for the protein enrichment of AP in SSF and SmF. The fungi secrete the cellulolytic enzymes resulting in release of sugars which were subsequently converted to protein rich biomass by yeasts. Solid-state culturing resulted in a 200% increase in protein enrichment when the combination of *C. utilis* and *A. niger* was used. Zheng and Shetty [186] utilized AP to produce a food rich in proteins by employing the fungus *Rhizopus oligosporus*. The results indicated that high moisture content results in an increase in mycelial production, consequently interfering in the transfer of oxygen. By contrast, low levels of moisture reduce fungal growth.

Villas-Bôas et al. [175] conducted a study to select biological treatments and conditions for the bioconversion of AP by *Candida utilis* and *Pleurotus ostreatus*, either individually or sequentially, into an enriched substrate with increased digestibility for use as ruminant feed. When the pomace was treated with *C. utilis*, there was an increase of 100% in the level of crude protein and mineral content 60%. Additionally, a reduction of up to 97% in the content of free sugars after supplementation of substrate with ammonium sulfate (10 g/l) in the substrate after 6 days of incubation was recorded. On the other hand, after optimization of parameters, sequential fermentation with *C. utilis* and *P. ostreatus*, the crude protein content was increased by 500% after a long incubation period of 60 days along with substantial increase in the mineral level. However, in the case of *P. ostreatus* alone, the level of free sugars increased after the fermentation due to degradation of hemicellulose and pectin and the material exhibited lower digestibility. Similarly, Albuquerque et al. [7] employed the filamentous fungus *R. oligosporus* in the protein enrichment of AP, and their results showed that the soluble protein content increased 5-fold in the best culture conditions.

3.8.5. Fish feed

Rapid expansion of human populations resulted in increased food production including fish production over recent years. However, due to high feed costs which represent from 50% to 80% of the total production cost, there has been rising interest in alternative sources of proteins in diets to reduce feed costs [59,116]. Agro-industrial residues are regarded as promising raw material for the production of SCP. Currently, due to lack of proper handing and utilization methods, these wastes are released directly into the environment having a serious impact. The biotechnological employment of these residues leads to the production of food with an excellent nutritional profile [25]. The addition of AP fermented with filamentous fungus to diets for fish may be a way of giving added value to this agro-industrial residue, reducing feed costs and environmental consequences.

In an attempt, Vendruscolo et al. [173] investigated the protein enrichment of AP by *G. butleri* through solid-state cultivation and application of fermented AP as feed for tilapia fry (*Oreochromis niloticus*). Factorial experimental design was used for the assessment of culture conditions to determine the effects of the source of nitrogen, initial moisture, and granulometry on the protein enrichment of AP. The best conditions obtained were with urea (5% w/w), initial moisture of 70% and granulometry in the range from 0.85 to 1.70 mm, producing 19.63% of soluble protein. The fish fed on the diet containing treated AP presented an increase of 44% in body mass, demonstrating that biologically transformed AP may represent an excellent food supplement. The increase of 3.2 times in the level of soluble proteins allows the use of this material as a food supplement in animal diets, partially replacing the feed with a view to reducing costs. Moreover, the applicability of AP after biological treatment does not need to undergo further secondary operations, except for a reduction in the moisture content and particle size for storage and its addition to conventional feed.

Devrajan et al. [36] conducted a study to utilize AP in an economical and effective way. A feed was developed by SSF using sequential interactive co-culture of *C. utilis* and *Kloeckera*. After recovery of ethanol, the left-over residue was dried. Feeding experiment was conducted in white albino rats before and after reconstitution of AP feed in the choice and no-choice study. Feeding of AP feed in the rats before reconstitution indicated that AP feed was acceptable neither in fermented nor in unfermented form. In the no-choice study both in 100% fermented and unfermented AP group feed intake decreased continuously resulting in death of rats apparently due to decreased digestibility owing to high fiber content of AP. Moreover, fermented or unfermented AP based feed had lower digestibility and efficiency of conversion than the standard rat feed and resulted in negative growth rates in all the feed groups. The results of choice study were similar with the no-choice study. However, incorporation of fermented AP into standard rat feed in the ratio of 1:1 gave better acceptability and digestibility. The results showed that reconstituted feed with 10% jaggery, 2% groundnut oil, 0.01% mixed flavor and 1% salt was the most acceptable.

3.8.6. Insect diets/biocontrol formulation preparations

Codling moth (CM), *Cydia pomonella* (L.) (Lepidoptera, Tortricidae), is an agriculturally important polyphagous pest affecting a wide variety of fruits, such as apple, pear, prune, walnut, almonds, peach, quince and, hawthorn in the temperate regions all over the world. The damage inflicted on fruit can be considerable with up to 80% of apples and up to 60% of pears infested on apple and pear plants if left untreated. Conventionally, the control of infestation of the fruits by CM has been carried out by the use of chemical pesticides (azinphos-methyl, commercial name Guthion) with adverse impacts, such as loss of natural enemies and pollinators, insecticide residues and environmental consequences [84].

However, due to environmental consequences of chemical pesticides, farmers have been looking for new safer technologies to keep CM populations below economic levels. Various technologies are currently practiced, such as the use of selective synthetic insect growth regulators (IGRs), mating disruption used alone or integrated with other cultural practices (post-harvest fruit removal, tree banding to catch over wintering larvae), “attract and kill”, and biological control agents, such as the use of *Bacillus thuringiensis* (Bt) [22]. However, use of these technologies shows little promise for CM control owing to resistance development.

At this juncture, exploitation of baculovirus can serve as a savior as it gives outstanding results even when the population pressures are high [105,9,164,178]. The baculoviruses-based biopesticides are prepared by infecting baculoviruses into CM larvae grown on specific diets. However, the cost of these diets is very high, limiting the growth of biopesticides at commercial scale. The baculoviruses constitute 70% of the biopesticides being used in North America and also represent 3% of the total biopesticide used for CM control [154].

Insect diet is mainly comprised of synthetic or semi-synthetic nutrients from plants or animals that are vital for insect nourishment [8,31]. Insects are able to synthesize some of the nutrients desired for their growth while others are exclusively provided by the diet [28]. In fact, the increasing demand for a large number of reared CM insects has mandated the development of more efficient and economical methods of its production. The rearing of these insects on artificial media rather than on their host plants is advantageous [31] due to the controlled environmental conditions and moreover, the percent yield is higher. In the overall biopesticide production process, 40% of the total cost is incurred towards the production of artificial larval diet. Existing larval diet contains essential sources, such as water, microbial growth inhibitors, sources of protein, carbohydrate and lipid, vitamins, salts, minerals and sterols. However, these diets are costly due to the use of synthetic ingredients [109] and adds to the overall cost economics of biopesticide production. However, some of these nutrient ingredients could be replaced by ubiquitous AP and APS which can help to reduce the effective production costs. Recently, a study demonstrated the potential of APS for preparing CM larvae diets [71]. The rheological study of the diets using the agro-industrial wastes (APS and brewery wastewater) was carried out in order to obtain a diet most adapted/efficient to supply nutrients for growth of CM larvae. The results demonstrated the nutritive capacity of APS waste as indispensable and important as replacement or in supplementation with other ingredients, such as soya flour, wheat germ, and yeast extract of the standard diet for the breeding of codling moth larvae. These wastes do not present risks to the environment and CM due to relatively lower concentration of different contaminants in particular heavy metals, such as Cd and Pb and coagulants, such as Al and Fe, which can be toxic at high concentrations [121,138]. In fact, a study has demonstrated that agro-industrial wastes can be used as supplement without any amendment for nourishing the animals [123].

The use of agro-industrial wastes, such as AP and APS, as diet supplements is a novel concept of sustainable use into the larval diet formulations. The economical diet development will have a broader application as the same can also be utilized for rearing other types of insects, such as cabbage looper, gypsy moth, Bertha armyworm, spruce budworm, cotton armyworms, hemlock looper, and tussock moth which have agricultural and economical importance. Moreover, APS can be used as a potential substrate to produce widely used *Bacillus thuringiensis* formulations.

4. Current AP management scenarios

Apple industry wastes have low nutritional value and their high biodegradability causes environmental hazards. A typical apple processing industry generates 30–40% AP and 5–10% sludge (liquid waste obtained after clarification) [38]. Generally, this waste is used as a source of crude animal feed by the farmers. Considering production of AP in huge quantities [17,400,910–20,881,092 tons AP and 3,480,182–6,960,364 of APS] only 20% is retrieved as animal feed and the rest 80% goes to landfill or composting sites which results in release of enormous quantity of GHGs. The inefficient management of apple industry wastes results in significant GHGs emissions [118]. The contribution of the waste management and disposal sector accounts for up to 4% of the various anthropogenic GHGs emissions [16]. Various stages of the management of solid wastes including collection, transportation, and disposal are generally followed by the release of GHGs, such as carbon dioxide, methane, and N_2O. These gaseous components by virtue of their physical properties contribute to the GHGs effect. Meanwhile, the increase in the concentration of these gases contributes to the global warming phenomenon.

Recently, studies were conducted to evaluate the GHGs production through different AP management strategies in Quebec [70]. Different strategies of AP management comprise incineration, landfill, composting, SSF for production of high value-added products, such as enzymes, organic acids, ethanol, among other products and animal feed. This study was unique as it discussed the GHGs emission analysis of AP waste management strategies and repercussions of value-addition of AP in terms of its sustainability using life cycle assessment (LCA) model. The results of the analysis indicated that, among all the AP management sub-models for a functional unit, enzyme production by SSF was the least polluting option of the environment in terms of GHGs emissions (906.81 tons CO_2 equivalent per year), followed by animal feed (963.38 tons of CO_2 equivalent per year), incineration (1122.10 tons of CO_2 equivalent per year), composting (1273.00 tons of CO_2 equivalent per year) and landfilling (1841.00 tons of CO_2 equivalent per year). The assessment and inventory of GHGs emissions during SSF gave positive indications of environmental sustainability for the use of this strategy to manage AP and other agricultural wastes, particularly in Quebec, Canada, and can also be extended to other countries. In fact, the option of high VAPs formation by SSF technique which was proven to be the effective method for reducing GHGs emissions has been tested as a resourceful option to synthesize various high-value products and at the same time result in efficient and sustainable management of the wastes.

5. Conclusion and future prospective

Apple industry wastes are produced in abundant quantities every year. Large quantities of these wastes are generally dumped in landfills which results in environmental pollution jeopardizing the life of flora and fauna. However, recent developments in fermentation and bioprocess technology provide promising alternatives for the biotransformation of these abundant wastes to high value products. The processes for value addition of these wastes can be integrated in the currently operating industries or separately as small scale units. The integrated processes will provide economic advantages to the industries with production of high value products. This will also curtail the environmental pollution and waste treatment cost for industries which they incur for the safe disposal of these wastes.

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